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Xenobanking has been used to successfully maintain and improve the function of stored kidney grafts for future allotransplantation. At present, the routine period of storage used experimentally is arbitrarily 3 days, a period twice that seen with the systems of organ perfusion in current use in many laboratories. With our present means of immunosuppression, i.e., lethal total body irradiation, the storage time can usually be extended to 10 or 11 days, after which termination occurs due to the death of the intermediate host. This provides a period of approximately 10 days' storage time which is a significant improvement over our presently available methods.

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Xenobanking for Organ Preservation

By Kenneth W. Sell and Jeffrey L. Benjamin

Organ preservation plays a key role in clinical renal transplantation.[1,2] Storage methods such as perfusion,[3] hypothermia,[4] and hyperbaria[5] have been examined in the laboratory and each has been used for the preservation of human organ grafts with reasonable, sometimes even encouraging, results.[6-8] In this paper, we will attempt to discuss another method of organ storage that has been used experimentally but has not yet been applied to clinical transplantation. This involves the use of a xenogeneic intermediate host, and has been termed "xenobanking."[9,10] The first question that might be asked is: Why should we examine a new method such as xenobanking for organ preservation? The most compelling answer is that no current method of storage offers a perfect system for organ banking. For example, the best clinical results have been achieved with kidneys that have been perfused by the in vitro method developed by Belzer.[3] The features of this method have now been repeatedly tested and verified in many transplantation centers.[11] However, even this system has limitations and provides only a few days of storage in the experimental animals, with failures beginning to be noted after 72 hr.[12] Clinically, this perfusion system has been used for the preservation of kidneys for little more than 2 days.[13] While it is possible that perfusion methodology may extend the period of storage, there does not appear to be the likelihood of an imminent breakthrough in this technique.

The ideal organ preservation system would, of course, be one that identically matches the normal physiological environment of the intact functioning animal or man. Such a system might even provide for repair and recovery of a damaged organ prior to definitive transplantation.[14] At the very least, xenobanking should allow us to carefully assess the quality of function of an organ being considered for grafting, and, additionally, would allow sufficient time, in excess of that now available, to ensure careful donor-recipient matching and transplantation, with surgery scheduled on an elective basis.

It has already been shown that organs stored in an intermediate host can function successfully after reimplantation into an autogeneic or allogeneic recipient. Ackerman[15] was able to preserve dog kidneys for 24 hr without immunosuppression, and for up to 5 days with immunosuppression in an allogeneic intermediate host. In both cases, the kidneys were reimplanted successfully either into the original donor or into a third dog immunosuppressed with Imuran, prednisone, and actinomycin. In each case, the kidneys functioned well during the entire 14-day period of observation. We have likewise been able to verify the feasibility of renal allograft storage in the primate. In these studies, the kidney was removed from a baboon, implanted into a

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second baboon that had been immunosuppressed by lethal irradiation, and then reimplanted after 5 days into the original donor. Recovery was prompt and function returned early. More than 6 mo later, the baboon had excellent renal function and biopsies showed no residual damage.

The principle of allograft storage in the human was first made feasible when Hume was able to show that a kidney graft attached extracorporeally to a patient with renal disease could function.[16] The intent of this procedure was to assist the failing renal function of the patient. Nevertheless, the result showed that a kidney could be maintained as an allograft. While the benefits of this early renal transplantation effort were transitory and therefore hard to evaluate, it served as a prelude to the later work of Lavender who reported the successful function of a human kidney attached to an extracorporeal shunt.[17] This kidney was maintained inside a plastic container and functioned for 25 days before undergoing acute vascular rejection. It not only functioned physiologically, but in some respects allowed clinical improvement in the patient's renal disease.

The use of allobank storage for human renal transplantation would seem to have limited applicability, however. Not many patients would want to serve as an intermediate "bank" for the storage of an organ which might then be transplanted to another human after tissue typing and cross-matching had been completed. In addition, the temporary attachment of a kidney to a human patient might result in either sensitization of the patient or the deposition of the intermediate host's preformed antibody in the kidney. Nevertheless, this technique could provide a means for quickly assessing whether a potential recipient had preformed antibody directed against the tissue graft. The acute rejection of an externally attached kidney would be far preferable to rejection of that same kidney at the time of intraabdominal implantation.

A more ideal system would allow the storage of a human organ in some other primate, who would thus serve as a more readily available, if not more willing, intermediate host. Xenobanking, however, provides many problems of its own. These include both the technical and immunological difficulties that are encountered in repeated xenotransplantation. We will attempt to discuss the problem under several topics including (1) spontaneous acute rejection between species, (2) an attempt to provide the best method of immunosuppression in the intermediate host, (3) evaluation of a potential recovery-repair of an organ during a period of storage in the intermediate host, and (4) evaluation of the difficulty encountered on reimplantation of the graft into the original animal, including a description of the immunological events that are apparently initiated by the addition of xenogeneic antigen from the intermediate host to the stored tissue graft. The last point includes the fact that this adsorption of xenogeneic antigen results in the initiation of an immunological response in the final recipient directed against these antigens.

Results

Acute Rejection of Xenografts-Natural Antibodies

The first problem to overcome in the use of a xenobank system for storage of kidney grafts is the hyperacute rejection often seen when kidneys are exchanged between different species. The severity of the rejection is accentuated if the donor and recipient are of a distant genetic relationship. For instance, a graft of a cat kidney to a dog results in hyperacute rejection and cessation of blood flow within 30 min.[18] When donor and recipient are of the same species, there is usually no acute rejection, when the cross-match is negative indicating that no cytotoxic preformed antibodies are present. In some human patients, however, the presence of anti-A or

anti-B red blood cell antibodies can lead to rejection of grafts that contain these antigens. In other patients, preformed antibodies can be produced due to previous blood transfusions, previous pregnancies, or previous tissue transplantation. In each instance, the presence of these antibodies may cause acute rejection of the tissue graft. Nevertheless, in general, kidney grafts exchanged between members of the same species are not subject to acute rejection. The object in the xenobank system, therefore, is to select closely related species who, like members of the same species, will have very low levels of natural cross-reactive antibody and therefore will not acutely reject the xenografted organ.

In an attempt to set up a xenobank system involving primates, a successful combination was shown to exist between the macaque monkey and the baboon. Sera obtained from each of the species tested against the lymphocytes of the opposite species showed only low levels of naturally occurring antibody. For instance, we have shown[22] that seven out of eight macaques produce cytolysis of baboon lymphocytes at titers of 0–16 and only one shows a titer of 1:32. These titers were sufficiently low as to minimize the chance of acute rejection of the xenografted organ. However, it should be noted that, even in these closely related species, the cellular rejection response is accelerated and seems to occur at a faster rate than is ordinarily seen in renal allografts. For instance, in five primate xenografts, performed without immunosappression, round cell infiltration was shown to occur within the first 24 hr in all and the urine flow ceased by 4–5 days.[10]

With the proper selection of closely related species, it is possible to overcome the first hazard encountered in a xenobank storage system: i.e., prevention of acute rejection due to preformed antibodies. The next step then is to control the cellular response against the xenograft.

Table 1.—Effect of Immunosuppression on Xenograft Survival as Measured by BUN Levels[a]

Days Post-grafting	Untreated Controls	800 Rads Lethal Total Body Irradiation	Treated With Azathioprine 10 mg/kg/day Prednisone 10–14 mg/kg/day
0	30	30	20
2	40	35	40
4	110	65	60
6	200	130	50
8	300	130	40
10	240	160	30

[a] All values represented as milligrams per 100 ml.

Immunosuppression of the Intermediate Host

While the acute rejection of the monkey kidney stored in the baboon is not the problem, there is a rapid and destructive immune cellular response. Attempts to control this delayed hypersensitivity by use of azathioprine at a dose of 10 mg/kg daily and prednisone at a dose of 5–7 mg/kg twice daily were not effective[11] (Table 1). While several other cytotoxic agents or antilymphocyte serum may have been tested in this respect, our laboratory elected to use a system which provides total immunosuppression, namely lethal total body irradiation. Eight hundred rads received from a standard 300 kV maxitron source proved to be 100% lethal in baboons within a period of 11–13 days. In 13 irradiated animals, the lymphocyte count fell to less than 25% of normal levels within 38 hr.[10] This form of immunosuppression, however, necessarily limited the period of organ storage to the survival time of the host. It was possible, nevertheless, to maintain one xenobanked kidney in a viable state for 11 days, during which there was minimal evidence of mononuclear infiltration or of any other deleterious effect. Biopsy specimens taken at 5 days in eight xenobanked kidneys showed minimal cellular infiltrate in only one kidney, and otherwise immunological damage was absent.

BUN levels and serum creatinine rose early after transplantation to the xenogeneic intermediate host, but, within 7 days, creatinine levels had returned to near normal and BUN levels which had reached a peak of over 60 mg/100 ml had fallen to less than 50 mg/100 ml. At this time, biopsies revealed a reversal of early tubular changes and a return of renal morphology to normal.

Successful Reimplantation of the Xenobanked Organ as an Autograft

Early attempts in our laboratory to xenobank primate hearts and kidneys were only partially successful.[10,18] Although total body lethal radiation provided adequate immunosuppression of the intermediate host, attempts to reimplant the stored organ were largely fruitless. Two explanations for this seem possible after an appraisal of these early results: a technical flaw in the procedure or an early immune response preventing adequate graft function. Benjamin has recently shown that both explanations were pertinent to the problems encountered after reimplantation.[19] Changes in technique, including the use of slightly larger macaque monkeys (*Macaca speciosa*), exceedingly careful renal dissection, as suggested by Collins et al.,[20] to prevent microcirculatory collapse, and omission of the gravimetric perfusion previously used on extirpation of the donor kidney, were all carried out. In addition, because of the possibility of early postoperative morbidity and depressed renal function, contralateral nephrectomy was delayed for 7 days. With these changes, we have been able to successfully reimplant[16] macaque kidneys after a 5-day period of xenogeneic storage. Once successful reimplantation was possible, we were further able to document an immune rejection process in eight of these kidneys that were reimplanted as autografts.[21] These kidneys which demonstrated excellent function while in the baboon intermediate host, upon reimplantation into the original donor, stimulated a cellular immune response in all eight (Fig. 1). Biopsies taken at 24 hr and weekly intervals thereafter up to 1 mo and subsequently at irregular intervals up to 6 mo showed that this lymphocyte infiltrate was transitory. The sequence usually seen was first a periglomerular, periarteriolar mononuclear infiltrate within 24 hr, accompanied by a significant decrease ($p < 0.01$) in peripheral lymphocyte counts from an average of 3700 to 1500 per cu mm. Simultaneously, a decrease in C3 levels was also noted, but its significance was clouded by the fact that complement levels were noted to drop after control renal allograft procedures, but not as profoundly, nor for as long a time. The infiltrate reached a peak at 7–10 days in all animals and thereafter gradually faded (Fig. 1), usually disappearing by 4 wk (Fig. 2). Accompanying changes in the glomerular basement membrane and vascular tufts did not, however, completely revert to normal until 6 mo postreimplantation. This data provided the first evidence that the xenobanked organ was modified, probably by the acquisition of baboon host antigen, thereby stimulating a cellular, and a humoral response of a monkey against

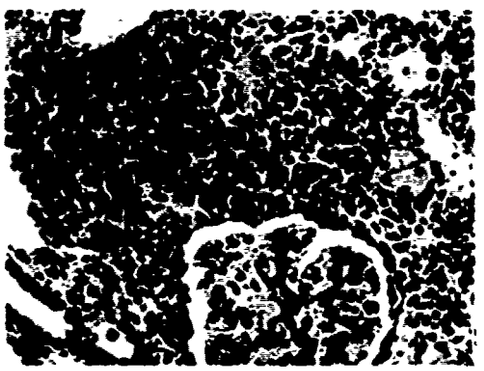

Fig. 1.—Mononuclear cellular infiltrate seen in macaque kidney autograft 1 wk after reimplantation of an organ which had previously been stored for 5 days as a xenograft in a lethally irradiated baboon.

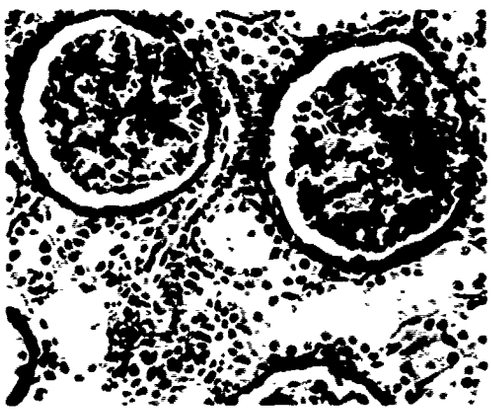

Fig. 2.—The same macaque kidney autograft 4 wk after reimplantation. The mononuclear infiltrate has cleared and morphological repair, though incomplete, is now evident.

its own kidney. That there was indeed a humoral response received further support when the serum of the final recipient was tested against baboon lymphocytes. A rapidly rising titer of cytotoxic antibody from an average of 1:16 to over 1:512 was noted, which peaked by day 21 postreimplantation with a fall to near pretransplant levels by day 30 (Table 2). A more detailed explanation of this phenomenon is expected from immunofluorescent studies now nearing completion.

Successful Reimplantation of Xenobanked Organ as Allograft

Four xenobanked kidneys that were reimplanted as allografts survived for 19–30 days compared to control allograft survival of 7–19 days.[22] This occurred despite a relentless and progressive mononuclear infiltrate in the kidney, accompanied by high serum levels of antibaboon lymphocytotoxic antibody up to 1:2048. Anti-macaque antibody, which might be expected to occur in a macaque renal allograft, was not detectable in lymphocytotoxic tests of recipient serum against donor lymphocytes; however, such antibody might be absorbed by the kidney and therefore not be readily detectable in the serum of the macaque recipient. In addition, it might be an enhancing antibody and not active in complement-dependent test methods.

Table 2.– Macaque Antibaboon Lymphocytotoxicity Titers Following Reimplantation of a Xenobanked Autograft

Days Postreimplantation	Lymphocytotoxicity Titer*
0	0–1:12
3	0–1:16
7	1:64–1:2048
10	1:128–1:2048
14	1:128–1:2048
21	1:4–1:64
28	0–1:8

* Rabbit complement used full strength. Data represents eight autografts.

Discussion and Summary

Xenobanking has been used to successfully maintain and improve the function of stored kidney grafts for future allotransplantation. At present, the routine period of storage used experimentally is arbitrarily 5 days, a period twice that seen with the systems of organ perfusion in current use in many laboratories. With our present means of immunosuppression, i.e., lethal total body irradiation, the storage time can usually be extended to 10 or 11 days, after which termination occurs due to the death of the intermediate host. This provides a period of approximately 10 days' storage time which is a significant improvement over our presently available methods.

Perhaps better means of immunosuppression could provide an intermediate host that could serve as a xenobank for a longer period of time. For instance, antilymphocyte serum has been shown to be relatively potent when used to suppress xenogeneic rejection responses.[23] Of course, if further investigation reveals a method of immunosuppression that would indefinitely prolong organ storage, we would have developed a technique that would make xenobanking unnecessary. In this instance, it would then be possible to transplant a primate organ

to man and obviate the need for human organ storage.

Data has been presented to suggest the acquisition of baboon antigen by the stored monkey kidney during its period of residence in the baboon intermediate host.[21,22] This baboon antigen apparently stimulates a cellular rejection response when returned to the organ donor. In addition, a serological humoral antibody response was also noted in the donor after reimplantation of his xenobanked organ. However, the studies show that both the cellular and humoral response to the xenobanked autograft is a temporary, nonprogressive, and totally reversible process.

Perhaps more important is the suggestion that xenobanked organs have a reduced, masked, or absence of antigenicity. A monkey kidney graft stored in a baboon xenobank, when subsequently implanted into a second monkey, may show a survival time of up to 30 days compared to normal survival times of 7–19 days in untreated monkey renal allografts that are not stored. There are several possible explanations for the prolonged survival of these allografts. (1) Perhaps, as has been suggested by Guttman[24] and others,[25] the lymphocyte is the main source of antigen in a kidney graft. If this is the case, then these cells which are active and motile may in great part be washed out or actively migrate from the graft during the storage period. Upon subsequent reimplantation of the stored allograft, the recipient would respond against xenogeneic antigen acquired by the graft, but would only slowly develop an allogeneic response against the kidney that had been depleted of macaque lymphocytes. (2) Macaque alloantigens may be blocked or covered by adsorbed baboon antigens. As the baboon antigens are removed, the macaque antigens may then be uncovered to stimulate a delayed allograft reaction. (3) The prolongation may be due to enhancing antibody. Validation of this possibility would require identification and elution of a protective antibody from the graft.

Xenobanking has provided an intriguing model for the study of problems inherent in organ storage, and of those present in xenotransplantation in general. In so doing, it will hopefully furnish answers to important questions posed by attempts at long-term clinical organ preservation and cross-species grafting.

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